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RESEARCH ARTICLE

Swimming performance of a freshwater fish during exposure to high carbon dioxide

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Abstract

Deterring the spread of invasive fishes is a challenge for managers, and bigheaded carp (including bighead and silver carp, *Hypophthalmichthys* spp.) are invasive fish that have spread throughout large portions of the Mississippi River basin and threaten to invade the Great Lakes' ecosystem. Studies have shown that elevated levels of carbon dioxide gas (CO_2) have the ability to act as a nonphysical fish barrier, but little work has been done on the efficacy of CO_2 to deter fish movement in flowing water. An annular swim flume was used to measure U_{burst} and sprint duration of the model species largemouth bass (*Micropterus salmoides*) across a range of pCO_2 levels (< 400 μatm [ambient]; 10,000 μatm; 50,000 μatm; and 100,000 μatm). This species was tested as a proxy because of the likelihood of a similar CO_2 response being produced, as well as constraints in obtaining and housing appropriately sized Asian carp. A significant decrease in U_{burst} swimming occurred when exposed to 100,000 μatm. No effects on sprint duration were detected. In both swimming tests, 15% of fish lost equilibrium when exposed to 50,000 μatm pCO_2, while 50% of fish lost equilibrium when exposed to 100,000 μatm. Together, results define target levels for managers to impede the spread of largemouth bass and potentially other invasive freshwater fishes, helping guide policy to conserve aquatic ecosystems.

Keywords Barrier · Climate change · Hypercarbia · Invasive species · Swimming performance

Introduction

Freshwater ecosystems vary widely with respect to dissolved carbon dioxide (CO_2) and are often supersaturated (Cole et al. 1994). With continually rising atmospheric CO_2 levels and other environmental changes (e.g., higher precipitation rates; Butman and Raymond 2011), some freshwater ecosystems are becoming even more hypercarbic and are acidifying (Weiss et al. 2018); however, which water bodies and the rate of change has not been determined (Hasler et al. 2016). The impacts of high dissolved CO_2 and weak acidification on aquatic biota are largely unknown (Hasler et al. 2016, 2018); however, some studies on freshwater fishes and invertebrates have been completed. Broadly, high CO_2 is known to have several behavioral and physiological effects on freshwater fishes (e.g., Heuer and Grosell 2014; Tierney 2016), but the exact outcomes vary and likely are dependent on CO_2 level and length of exposure (Kates et al. 2012). Lab studies have shown several trends, including loss of predator awareness (Tix et al. 2017), altered behavior (Cupp et al. 2016), and loss of equilibrium (Kates et al. 2012) when exposed to elevated CO_2. Due to the suite of physiological and behavioral effects that hypercarbia has on fishes, the use of plumes of intentionally elevated CO_2 to prevent the movement of invasive fishes (e.g., bigheaded carp) has been explored (Noatch and Suski 2012; Treanor et al. 2017).

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Though there is variation in how fish respond to prolonged exposure to high CO_2, avoidance of high CO_2 seems to be robust across freshwater fishes (Cupp et al. 2016; Dennis et al. 2016a; Kates et al. 2012) and across magnitude of exposure (Donaldson et al. 2016), likely as fish seek out improved water quality to avoid potential costs from CO_2 exposure. For this reason, injection of CO_2 into canal locks is being considered as a method to limit the distribution of invasive species in the Mississippi River and elsewhere (Treanor et al. 2017), particularly bigheaded carp (*Hypophthalmichthys* spp.; Noatch and Suski 2012). Additionally, this potential technology could be used for applications with native fishes as well. Fish possess chemoreceptors localized on the gills that sense increasing levels of ambient CO_2 (Perry and Abdallah 2012), and different species (invasive carp and natives) often react similarly to CO_2 exposure (Cupp et al. 2016; Kates et al. 2012). Specifically, Kates et al. (2012) showed that silver and bighead carp (*H. molitrix* and *H. nobilis*, respectively), largemouth bass (*Micropterus salmoides*), and bluegill (*Lepomis macrochirus*) all showed similar responses to elevated CO_2 including elevated stress hormone levels, modified ion balance, increased ventilation, and loss of equilibrium all occurring at similar levels across different species. Thus, injecting plumes of CO_2 into fish habitat will adversely affect both native and non-native fishes.

Exposure to CO_2-rich plumes intended to prevent the movement of invasive fish may have impacts on non-target species, including economically important sport fish. A possible outcome of exposure to high CO_2 may be reduced swimming performance due to lack of coordination (Yoshikawa et al. 1994), a central phenotype in determining fish fitness (Plaut 2001). Loss of equilibrium and swimming coordination is thought to be caused by CO_2 moving into the brain and affecting energy processing (Yoshikawa et al. 1994). As swimming is either fueled by oxygen (aerobic) or glycolytic products (anaerobic), the effect CO_2 has on swimming performance may be context-dependent, though ultimately controlled by the tolerance of the brain to maintain coordination. Furthermore, understanding how fish not only experience altered behavior but also altered swimming performance in hypercarbic environments can have ecological and management implications. However, a thorough investigation of the effects of elevated CO_2 levels on various swimming strategies (here, burst and sprint) in freshwater fishes has yet to occur. This information is essential in understanding how fish will interact with an increasingly hypercarbic environment and has high potential to steer future management strategies and develop tools to prevent the spread of invasive fish.

To address these shortcomings, this study aimed to define (1) the interactive effects of pCO_2 and exposure duration on burst swimming performance in a freshwater fish and (2) the effects of elevated pCO_2 on sprint swimming performance, with an emphasis on barrier development in a flowing water environment. In assessing the impacts that elevated pCO_2 may have on both mid-range burst swimming and short-distance sprint swimming performance, an understanding of how exposure to a carbon dioxide barrier may impair the swimming performance of fishes attempting to traverse a CO_2 plume can be developed, and management recommendations can be made to maximize the effectiveness of a CO_2 barrier (including factors such as the size and velocity a barrier would need to be and target pCO_2 required to impair movement through it). Despite both burst and sprint swimming being primarily anaerobically fueled, we predict that the incorporation of some aerobic metabolism in a long (~ 30 min) burst swimming activity may cause burst swimming performance to decrease as pCO_2 increases, while sprint swimming will remain unaffected. This may occur due to a combination of the anesthetic properties of CO_2, as well as the potential impacts of pCO_2 on oxygen uptake and delivery in fish (Portner et al. 2004). However, this is largely unknown as the root drivers (central nervous system or muscles) leading to a breakdown in swimming abilities under hypercarbic conditions are still unclear.

Methods

Fish holding and husbandry

In November 2016, sub-adult largemouth bass (*Micropterus salmoides*) were purchased from a hatchery (Keystone Hatcheries, Richmond, IL; pond-raised, pellet-fed) and transported to the Aquatic Research Facility at the University of Illinois in Urbana, Illinois, where they were acclimated for 1 week in a 1200-L indoor holding tank. During this time, fish were fed pelleted commercial food (Purina Aquamax, St. Louis, MO) to satiation daily; however, food was withheld for 24 h prior to trials to ensure a post-absorptive state was reached before swimming trials (Roche et al. 2013). Water was sourced from a nearby earthen-bottom pond with natural vegetation, and 10% water changes were performed daily to maintain water quality. Both mechanical (Fluval 406 Canister Filter, Mansfield, MA) and UV filters (Vecton-4: V2 400 15 Watt UV Filter), along with supplemental aeration, were used to maintain optimum water quality. Dissolved oxygen (YSI ProODO, Yellow Springs Instruments, Irvine, CA, USA), temperature (YSI ProODO, Yellow Springs Instruments, Irvine, CA, USA), pH (WTW pH 3310 meter, SenTix probe, Germany), ammonia (LaMotte Company, Ammonia Nitrogen Kit no. 3351-02, Chestertown, MD, USA), total alkalinity (Hach Company, Titrator 16,900, kit 2272700, Loveland, CO, USA), and pCO_2 (GMT220 Infrared CO_2 meter, Vaisala, Vantaa, Finland) were monitored in the holding tank daily (Table 1). Largemouth bass were selected for this study due to the limitations in collecting and housing appropriately sized Asian

Table 1 Water quality parameters measured once daily in the 1200-L fish holding tank through the duration of experimentation. All values shown are mean ± 1 standard error

Temperature (°C)	Dissolved oxygen (mg L^{-1})	pH	pCO_2 (μatm)	Total alkalinity (mg L^{-1} of $CaCO_3$)	Ammonia (mg L^{-1})
20.0 ± 0.2	7.9 ± 0.2	8.1 ± 0.0	295.5 ± 37	158.6 ± 5.2	1.6 ± 0.3

carp. Expected similarities in CO_2 responses between these fishes are detailed in the discussion.

U_{burst} swimming

To quantify the effects of pCO_2 exposure on U_{burst} swimming, a swim tunnel (30 L swim tunnel respirometer, Loligo Systems, Viborg, Denmark) was used to perform a constant acceleration test wherein fish were forced to swim against an elevated current velocity in water treated with CO_2 (Reidy et al. 2000). The swim tunnel was calibrated using an in-line flow meter (HFA, Höntzsch GmbH, Waiblingen, Germany), which allowed us to relate motor speed to water velocity. After appropriate lab acclimation and starvation periods of 24 h, a single fish was netted from the holding tank, loaded into the test chamber of the swim tunnel, and acclimated for 10 min at a velocity of 0.5 body lengths per second (BL s^{-1}) (Gregory and Wood 1998) in water at ambient pCO_2. Concurrent with this, water in an external holding tank was treated to a target pCO_2 using the common method of bubbling compressed CO_2 gas through an airstone (Tix et al. 2016). The pCO_2 treatments used were < 400 μatm (ambient), 10,000 μatm, 50,000 μatm, and 100,000 μatm. The ambient treatment of 400 μatm falls within normal expected ranges for inland lakes and rivers (Cole et al. 1994). Additionally, similarly elevated pressures can induce physiological and behavioral responses in both largemouth bass and bigheaded carp, and also span the range of pressures that would be targeted for use in a non-physical fish barrier (Cupp et al. 2016; Dennis et al. 2016a; Donaldson et al. 2016; Kates et al. 2012; Noatch and Suski 2012), as well as in areas downstream of barrier applications where CO_2 may have dissipated relative to application sites. The use of multiple pCO_2 levels is useful for identifying mechanisms and stimulating hypotheses about how pCO_2 may impact fish biology (Heuer and Grosell 2014).

Following the acclimation period, water from the external holding tank at the pre-defined pCO_2 was pumped into the test chamber, and the swimming trial was initiated 15 s later. Pumping CO_2-rich water from the external holding tank caused water at ambient pCO_2 in the swim tunnel to be displaced, and the excess water exited the swim tunnel via an overflow tube in the top of the tunnel. The propeller in the swim tunnel ensured mixing within the swim tunnel, and preliminary trials indicated that water in the swim tunnel reached target pCO_2 after 15 s. Once target pCO_2 was reached in the swim tunnel, largemouth bass were forced to swim in elevated pCO_2 for either 30 s, 120 s, or 600 s, after which "fresh" water at ambient pCO_2 was flushed through the swim tunnel. These durations were selected because fish exposed to elevated pCO_2 for similar times have displayed both behavioral and physiological changes (Dennis et al. 2016a; Kates et al. 2012). Together, all possible combinations of the above CO_2 pressures (< 400 μatm [ambient]; 10,000 μatm; 50,000 μatm; and 100,000 μatm) and exposure durations (30 s, 120 s, and 600 s) were tested in a 4 × 3 full-factorial design for a total of 12 distinct treatments ($n = 8$ fish per treatment).

At the start of the swimming trial, water velocity was increased at a linear and continuous rate of 0.2 BL s^{-1} every minute (adapted from Reidy et al. 2000). Fish maintained swimming under these conditions for anywhere from 10–25 min. The swimming trial was concluded when the fish was no longer able to maintain swimming position in the current, evidenced by it becoming pinned against the downstream grate of the test chamber, at which point the water velocity was recorded as its U_{burst} swimming velocity (Gregory and Wood 1998; Reidy et al. 2000). Fish that lost equilibrium were easily distinguished from fish that were simply exhausted as they rolled and inverted just before contacting the rear grate, whereas fish that became exhausted before losing equilibrium remained vertically oriented and struggled to continue swimming after contacting the grate. If equilibrium loss occurred, this was noted and the water velocity was similarly recorded as an endpoint for U_{burst} swimming velocity. At either of these two endpoints, the motor was turned off and the fish was allowed to recover before being removed from the swim tunnel. All fish were measured and size did not differ across treatments ($n = 8$ fish per treatment, ANOVA: total Length, $F_{12,103} = 0.66$, $p = 0.784$). Water quality data for each swimming trial was also measured from the source header tank providing water to the swim tunnel (Table 2). Trials were always performed during daylight hours (typically between 08:00 and 16:00) at an average rate of 3 fish per day.

One additional experiment was performed during which fish were forced to swim in the tunnel without pumping ambient water in from a separate header tank. The purpose of this experiment was to define any impacts of pumping water into the swim tunnel on swimming performance. These methods and results are detailed in Supplementary Material and

Table 2 Water quality parameters measured during the U_{burst} *swimming* trials (**A**) ($n = 8$) and during the *sprint swimming* trials (**B**) ($n = 10$) in the external header tank that provided water to the swim tunnel. Water from the header tank was pumped into the swim tunnel for swimming trials, and header tank water replaced swim tunnel water in approximately 15 s (confirmed in preliminary trials using the same probe to measure pCO_2 of water exiting the swim tunnel outflow). Data shown are mean ± 1 standard error

pCO_2 treatment (μatm)	Exposure duration (s)	Temperature (°C)	Dissolved oxygen (mg L^{-1})	pH	pCO_2 (μatm)	Total alkalinity (mg L^{-1} of $CaCO_3$)	Titrated CO_2 (mg L^{-1})
A							
Ambient (< 400)	0	21.8 ± 0.4	6.88 ± 0.3	8.0 ± 0.1	137 ± 35	177.5 ± 3.6	15.8 ± 1.1
Ambient (< 400)	30	22.0 ± 0.3	7.0 ± 0.3	8.2 ± 0.1	362 ± 66	175.5 ± 3.3	13.4 ± 1.8
Ambient (< 400)	120	21.1 ± 0.3	7.4 ± 0.2	8.2 ± 0.1	212 ± 62	175.5 ± 4.0	13.8 ± 1.3
Ambient (< 400)	600	21.7 ± 0.4	6.8 ± 0.4	8.1 ± 0.1	287 ± 104	176.8 ± 3.7	14.8 ± 1.7
10,000	30	21.8 ± 0.4	6.9 ± 0.0	7.1 ± 0.1	10,965 ± 298	159.3 ± 4.2	30.9 ± 1.7
10,000	120	21.0 ± 0.6	6.8 ± 0.1	7.3 ± 0.1	10,103 ± 278	163.8 ± 4.6	34.6 ± 2.4
10,000	600	20.7 ± 0.6	7.0 ± 0.2	7.3 ± 0.1	10,778 ± 531	161.8 ± 3.4	34.6 ± 2.4
50,000	30	21.0 ± 0.6	8.0 ± 0.5	6.8 ± 0.1	49,116 ± 1031	173.0 ± 2.1	81.8 ± 5.0
50,000	120	21.2 ± 0.5	8.0 ± 0.5	6.8 ± 0.0	49,788 ± 413	175.5 ± 1.7	78.4 ± 5.1
50,000	600	21.8 ± 0.5	7.2 ± 0.3	6.8 ± 0.0	50,754 ± 770	174.5 ± 1.6	76.5 ± 5.0
100,000	30	21.8 ± 0.3	6.8 ± 0.1	6.5 ± 0.0	94,240 ± 1209	175.5 ± 2.3	121.9 ± 12.6
100,000	120	21.7 ± 0.3	6.9 ± 0.2	6.5 ± 0.0	97,575 ± 1113	174.0 ± 2.7	126.3 ± 11.9
100,000	600	21.5 ± 0.3	6.9 ± 0.1	6.5 ± 0.0	99,835 ± 1656	180.3 ± 3.0	160.0 ± 7.9
B							
Ambient (< 400)		22.6 ± 0.6	6.5 ± 0.2	8.2 ± 0.1	320 ± 107	200.0 ± 1.6	12.8 ± 1.9
10,000		23.6 ± 0.4	5.7 ± 0.1	7.4 ± 0.1	10,910 ± 226	208.8 ± 3.4	32.6 ± 1.7
50,000		24.0 ± 0.2	5.5 ± 0.1	6.7 ± 0.1	48,287 ± 813	205.8 ± 3.4	78 ± 1.8
100,000		24.3 ± 0.2	5.5 ± 0.1	6.5 ± 0.0	94,735 ± 1652	206.4 ± 2.4	150.2 ± 1.4

indicated no impact of pumping water from an external header tank into the swim tunnel on fish swimming performance, such that any changes to swimming performance can be attributed to the increase of CO_2.

Sprint duration

The impacts of exposure to elevated pCO_2 on sprint duration were quantified using the same swim tunnel and conditions as described above (fish were not re-used from the burst swimming trials). For these trials, however, following the 10-min acclimation to the swim tunnel at a velocity of 0.5 BL s^{-1}, water velocity was immediately increased to 4 BL s^{-1} to transition the fish into a sprinting gait (detailed in Reidy et al. 2000; Tierney 2011). At this time, CO_2-treated water at the same targeted pressures was again pumped into the swim tunnel using methods identical to those described above. CO_2-treated water was pumped into the swim tunnel 15 s before the trial was initiated and the velocity was increased, to ensure that the target level was reached in the swim tunnel before the trial began. Time to exhaustion for this sprint test was recorded when the trial ended, which was defined by one of two endpoints: when the fish was no longer able to maintain swimming position and became pinned against the downstream grate of the test chamber, or when the fish lost equilibrium and ceased swimming activity, again. At this point, the motor was turned off and the fish was able to recover. All fish were measured and size did not differ across treatments ($n = 10$ fish per treatment, ANOVA: total length, $F_{3,39} = 1.10$, $p = 0.361$). Water quality data were also measured from the source header tank providing water to the swim tunnel (Table 2).

Data analyses

A linear model was used to quantify the fixed effects of pCO_2, exposure duration, and the interaction between pCO_2 and exposure duration on U_{burst} swimming (a Tukey test was used post hoc). Similarly, the effect of pCO_2 on sprint duration was assessed using a one-way analysis of variance (ANOVA), with pCO_2 as the independent variable. All data were log-transformed prior to analyses to ensure that residuals from fitted models passed visual inspection for both normality and homogeneity of variances, and significance (α) was tested at the 95% confidence level (Zar 1984). Logistic regressions were used to analyze the effects of pCO_2 and exposure duration on loss of equilibrium during the U_{burst} swimming protocol and to analyze the effects of pCO_2 on loss of equilibrium during the sprint duration test. All statistical tests were completed in JMP Pro 11 (SAS Institute, Cary, NC).

Results

U_{burst} swimming velocity did not differ for largemouth bass exposed to pCO_2 levels of either 10,000 or 50,000 μatm relative to swimming velocity at 400 μatm (ambient) (Fig. 1a). However, when forced to swim in water treated to 100,000 μatm pCO_2, largemouth bass experienced a significant 30% decrease in U_{burst} swimming velocity relative to water at ambient and 10,000 μatm. The decrease in U_{burst} swimming velocity occurred independent of exposure duration (Fig. 1a; Table 3). In addition, fish lost equilibrium at a significantly higher rate as pCO_2 increased, and this loss of equilibrium was independent of exposure duration (Fig. 1b; Table 4). More specifically, U_{burst} swimming in water at either ambient pCO_2 or in water treated to 10,000 μatm did not cause any loss of equilibrium, and all fish were able to swim until exhaustion. When exposed to water at 50,000 μatm for 600 s, 15% of fish lost equilibrium prior to reaching exhaustion, while water treated to 100,000 μatm resulted in loss of equilibrium at rates of 12.5%, 25%, and 50%, respectively, for the different exposure durations (30 s, 120 s, and 600 s) (Fig. 1b; Table 4).

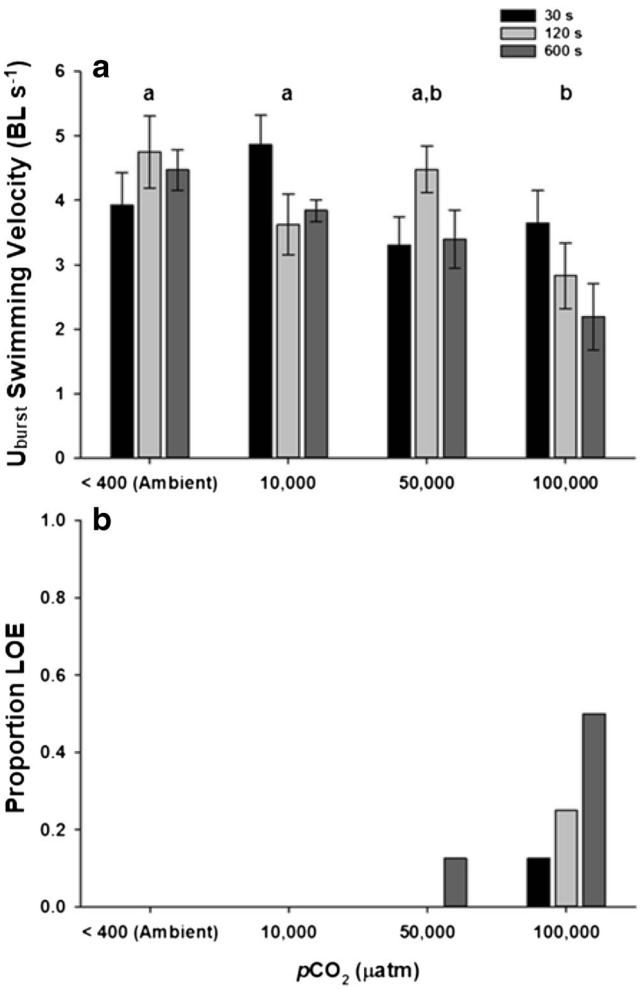

Fig. 1 U_{burst} swimming velocity (measured in BL s^{-1}) (**a**) and proportion of individuals that lost equilibrium (**b**) for largemouth bass (*M. salmoides*) exposed to a range of pCO_2 (< 400; 10,000; 50,000; or 100,000 μatm) for either 30 s, 120 s, or 600 s ($n = 8$ fish per treatment). Dissimilar letters (**a** and **b**) represent significant differences across pCO_2 treatments

Table 3 Results from a linear model used to analyze the effects of pCO_2 (ambient < 400 μatm; 10,000 μatm; 50,000 μatm; and 100,000 μatm) and exposure duration (30 s, 120 s, and 600 s) on U_{burst} swimming performance in largemouth bass. Significant results are shown in italics

Main effects	*df*	*F*	*p*
CO_2 pressure	*3*	*5.44*	*0.0018*
Exposure duration	2	1.19	0.3107
Pressure × duration	6	1.86	0.0969

Largemouth bass experienced no significant changes in sprint duration (measured as time to exhaustion) across any of the pCO_2 treatments (Fig. 2a; ANOVA, $F_{3,39} = 1.12$, $p = 0.296$). However, the proportion of fish that lost equilibrium significantly increased with pCO_2, similar to results from the U_{burst} swimming test. More specifically, none of the largemouth bass lost equilibrium during the sprint test at 400 μatm (ambient) or 10,000 μatm pCO_2, only 20% of fish tested lost equilibrium during the sprint test at 50,000 μatm, and 70% lost equilibrium at 100,000 μatm (Fig. 2b; Table 4).

Discussion

Largemouth bass only experienced an impairment in burst swimming performance at the highest levels of CO_2 tested, and this was independent of exposure duration. Specifically, U_{burst} swimming performance decreased by about 30% at 100,000 μatm relative to ambient pCO_2. When fish operate in areas where environmental pCO_2 is increased, fish blood is at risk of becoming acidified due to the influx of protons

Table 4 Results from a logistic regression used to analyze the effects of pCO_2 and exposure duration on loss of equilibrium (LOE) during U_{burst} swimming, and the effects of pCO_2 on loss of equilibrium during sprint swimming. Significant results are highlighted in italics

Measured variable	Main effects	*df*	X^2	*p*
LOE during U_{burst} swimming	CO_2 pressure	*1*	*15.33*	*< 0.001*
	Exposure duration	1	1.92	0.1658
	CO_2 pressure × exposure duration	1	0.58	0.4476
LOE during sprint swimming	CO_2 pressure	*1*	*19.57*	*< 0.001*

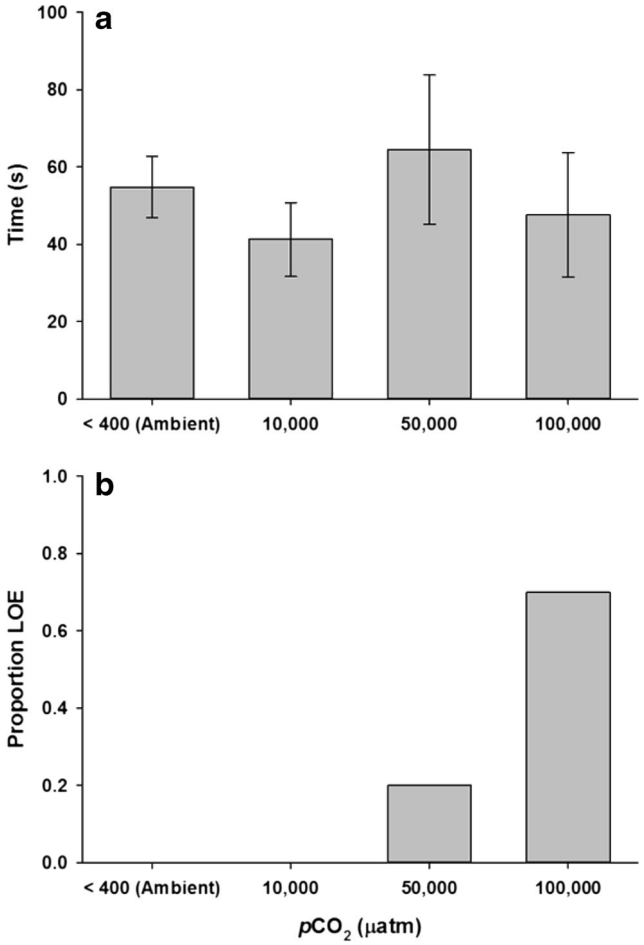

Fig. 2 Time to exhaustion during sprint swimming trials (measured in seconds) (**a**) and proportion of individuals that lost equilibrium (**b**) for largemouth bass during sprint swimming trials when exposed to a range of pCO_2 (< 400; 10,000; 50,000; or 100,000 μatm) ($n = 10$ fish per treatment)

(Janssen and Randall 1975). Fish are generally efficient acid-base regulators, however, and they can often maintain appropriate blood pH by either exporting protons or importing base (bicarbonate) (Evans et al. 2005). If acid-base regulating mechanisms are unable to maintain homeostasis under hypercarbic conditions, this can result in an impaired ability to transport oxygen due to reductions in hemoglobin efficiency explained by the Bohr and Root effects (Brauner and Randall 1996), which may impair certain swimming activities. While the present study tested burst swimming which is primarily anaerobically fueled, fish swam for as long as 30 min and may have employed some aerobic metabolism. The fact that we saw no impairment of burst swimming at the low pCO_2 treatment can indicate that largemouth bass may be able to successfully regulate acid-base status to maintain oxygen delivery to tissues, but are unable to maintain homeostasis above 100,000 μatm. Largemouth bass are robust at maintaining oxygen delivery to tissues in the face of external challenges with Furimsky et al. (2003) demonstrating that largemouth bass can maintain oxygen delivery to tissues despite ambient dissolved oxygen falling by 60%. Alternatively, if the effects of hypercarbia are being derived in the central nervous system, this may have a similar outcome as effects manifested in the muscles. Together, results from this study clearly show that elevated CO_2 impairs the ability of fish to burst swim, but only at pCO_2 above 50,000 μatm.

Largemouth bass also did not show any changes in sprint swimming performance when exposed to a range of CO_2 pressures. Specifically, sprint duration while swimming at 4 BL s^{-1} did not differ between ambient (< 400 μatm) and 100,000 μatm pCO_2. Sprint swimming, which is powered by white muscle, is often employed by fish for short duration, high-intensity behaviors such as capturing prey and avoiding predation (Domenici and Blake 1997), or making upstream movements through high-velocity flows. Sprint swimming is fueled by anaerobic glycolysis with energy substrates coming from stores within the white muscle (Dobson et al. 1987; Garenc et al. 1999). While Randall and Brauner (1991) suggest that sprinting activities are largely unaffected by changes in environmental conditions unless they alter muscle efficiency or energy stores, other work suggests that factors such as temperature (Batty and Blaxter 1992) and hypercarbia (Dennis et al. 2016a) can affect sprint swimming performance. More specifically, Dennis et al. (2016a) showed that sprint swimming performance by juvenile largemouth bass decreased when exposed to 120 mg/L CO_2. However, this decrease was after an exposure of 2 h that could have potentially led to partial anesthesia or altered acid-base physiology and hence a reduced swimming performance. Results from this study demonstrate that elevated CO_2 environments do not impair the ability of largemouth bass to perform sprint swimming.

During both burst and sprint swimming activities, largemouth bass lost equilibrium with increasing frequency as pCO_2 increased. The proportions of fish that lost equilibrium during trials were similar during burst and sprint swimming activities, occurring around 20% of the time at 50,000 μatm and up to 70% of the time at 100,000 μatm (depending on exposure duration). CO_2 is known to have anesthetic properties on fish and has previously been used as such in fisheries work (Gilderhus and Marking 1987). As environmental pCO_2 increases, the gradient between a fish's external and internal CO_2 is reduced or reversed, rendering the fish unable to excrete CO_2 (Janssen and Randall 1975). Fish are able to counteract this internal acidification by exporting protons or gaining bicarbonate, but this typically fails above 10,000 μatm (reviewed by Heuer and Grosell 2014). While the exact mechanism that causes fish to lose equilibrium is not well understood, movement of CO_2 across the blood-brain barrier and disrupting energy processing is likely the cause (Yoshikawa et al. 1994). In our study at pressures below 100,000 μatm, largemouth bass appear to have the ability to

maintain homeostasis as little equilibrium loss occurred. Once pressures of 100,000 μatm were reached, however, there was a significant increase in equilibrium loss independent of exposure duration, indicating that this pressure likely overwhelmed acid-base mechanisms. Together, results from this study clearly show that a CO_2-induced loss of equilibrium will frequently occur at 100,000 μatm pCO_2 during both burst and sprint swimming in largemouth bass.

Research from static ponds and tanks indicate carp will swim away from a CO_2 barrier, giving it potential to act as a "push-type" barrier that can repel fish from an area (Noatch and Suski 2012). As such, CO_2 is being considered as a barrier to deter the movements of invasive bigheaded carp in Illinois, with flowing water as a potential application site (United States Army Corps of Engineers 2014). Target pCO_2 for the barrier based on previous findings has been estimated to be around 30,000–50,000 μatm (Cupp et al. 2016; Dennis et al. 2016b; Donaldson et al. 2016). Deployment strategies will likely involve injecting CO_2 into a lock or creating a "wall" of CO_2 at a chokepoint such as a culvert or a lake inlet, with elevated pCO_2 presumably dissipating as water flows downstream. Our study shows that if fish decide to challenge a barrier and swim through it using either burst or sprint swimming, there would be little if any impairment to their swimming ability at pressures previously demonstrated to induce avoidance. However, a barrier could still impede passage of a portion of fish by inducing a loss of equilibrium, but this goal would necessitate a target of 50,000–100,000 μatm and would need to be paired with other technologies for complete deterrence. Future deployments could either rely on the barrier acting as a push-type deterrent at 50,000 μatm or raise the target pressure to 100,000 μatm to achieve equilibrium loss, thereby increasing the likelihood of immobilization. Thresholds for equilibrium loss need to be defined in other target fish species in flowing water, with a priority on potentially invasive fishes (e.g., bigheaded carp, ruffe (*Gymnocephalus cernuus*), and round goby (*Neogobius melanostomus*)).

Aquatic invasive species are problematic due to their negative impacts on ecosystem services and commerce and their ability to outcompete native species (Ehrenfeld 2010; Smith et al. 2015). More importantly, it is more difficult to eradicate invasive species from an area following establishment than to prevent them from spreading (McDermott et al. 2013). To date, prototype CO_2 barriers have proven effective as push-type detractors that exclude fish from target areas, and results from our study show that a carbon dioxide barrier will likely not impair swimming performance unless target pressures of 50,000–100,000 μatm are reached. Efforts should be made to better define the impairment to target species such as Asian carp and to begin scaling deployment efforts from lab to field trials in hopes of protecting the aquatic ecosystems that are at risk from invasive species.

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References

Batty RS, Blaxter JHS (1992) The effect of temperature on the burst swimming performance of fish larvae. J Exp Biol 170:187–201

Brauner CJ, Randall DJ (1996) The interaction between oxygen and carbon dioxide movements in fishes. Comp Biochem Physiol 113A(1):83–90

Butman D, Raymond PA (2011) Significant efflux of carbon dioxide from streams and rivers in the United States. Nat Geosci 4:839–842

Cole JJ, Caraco NF, Kling GW, Kratz TK (1994) Carbon dioxide supersaturation in the surface waters of lakes. Science 265:1568–1570

Cupp AR, Erickson RA, Fredricks KT, Swyers NM, Hatton TW, Amberg JJ (2016) Responses of invasive silver and bighead carp to a carbon dioxide barrier in outdoor ponds. Can J Fish Aquat Sci 74(3):297–305

Dennis CE, Adhikari S, Wright AW, Suski CD (2016a) Molecular, behavioral, and performance responses of juvenile largemouth bass acclimated to an elevated carbon dioxide environment. J Comp Physiol B 186:297–311

Dennis CE, Wright AW, Suski CD (2016b) Potential for carbon dioxide to act as a non-physical barrier for invasive sea lamprey movement. J Great Lakes Res 42:150–155

Dobson GP, Parkhouse WS, Hochachka PW (1987) Regulation of anaerobic ATP- generating pathways in trout fast-twitch skeletal muscle. Am J Physiol 253:186–194

Domenici P, Blake RW (1997) The kinematics and performance of fish fast-start swimming. J Exp Biol 200:1165–1178

Donaldson MR, Amberg J, Adhikari S, Cupp A, Jensen N, Romine J, Wright A, Gaikowski M, Suski CD (2016) Carbon dioxide as a tool to deter the movement of invasive bigheaded carps. Trans Am Fish Soc 145:657–670

Ehrenfeld JG (2010) Ecosystem consequences of biological invasions. Annu Rev Ecol Evol Syst 41(1):59–80

Evans DC, Piermarini PM, Choe KP (2005) The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol Rev 85:97–177

Furimsky M, Cooke SJ, Suski CD, Wang Y, Tufts BL (2003) Respiratory and circulatory responses to hypoxia in largemouth bass and smallmouth bass: implications for "live-release" angling tournaments. Trans Am Fish Soc 132:1065–1075

Garenc C, Couture P, Laflamme M-A, Guderley H (1999) Metabolic correlates of burst swimming capacity of juvenile and adult threespine stickleback (Gasterosteus aculeatus). J Comp Physiol B 169:113–122

Gilderhus PA, Marking LL (1987) Comparative efficacy of 16 anesthetic chemicals on rainbow trout. N Am J Fish Manag 7(2):288–292

Gregory TR, Wood CM (1998) Individual variation and interrelationships between swimming performance, growth rate, and feeding in juvenile rainbow trout (Oncorhynchus mykiss). Can J Fish Aquat Sci 55(7):1583–1590

Hasler CT, Butman D, Jeffrey JD, Suski CD (2016) Freshwater biota and rising pCO2? Ecol Lett 19(1):98–108

Hasler CT, Jeffrey JD, Schneider EVC, Hannan KD, Tix JA, Suski CD (2018) Biological consequences of weak acidification caused by elevated carbon dioxide in freshwater ecosystems. Hydrobiologia 806(1):1–12

Heuer RM, Grosell M (2014) Physiological impacts of elevated carbon dioxide and ocean acidification on fish. American Journal of Physiology- Regulatory, Integrative and Comparative. Physiology 307(9):1061–1084

Janssen RG, Randall DJ (1975) The effects of changes in pH and P_{CO2} in blood and water on breathing in rainbow trout. Salmo gairdneri Respiration Physiology 25(2):235–245

Kates D, Dennis C, Noatch MR, Suski CD (2012) Responses of native and invasive fishes to carbon dioxide: potential for a nonphysical barrier to fish dispersal. Can J Fish Aquat Sci 69:1748–1759

McDermott SM, Irwin RE, Taylor BW (2013) Using economic instruments to develop effective management of invasive species: insights from a bioeconomic model. Ecological Society of America 23(5):1086–1100

Noatch MR, Suski CD (2012) Non-physical barriers to deter fish movements. Environ Rev 20:1–12

Perry SF, Abdallah S (2012) Mechanisms and consequences of carbon dioxide sensing in fish. Respir Physiol Neurobiol 184:309–315

Plaut I (2001) Critical swimming speed: its ecological relevance. Comparative Biochemistry and Physiology Part A 131:41–50

Portner HO, Langenbuch M, Reipschlager A (2004) Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. J Oceanogr 60:705–718

Randall D, Brauner C (1991) Effects of environmental factors on exercise in fish. J Exp Biol 160:113–126

Reidy SP, Kerr SR, Nelson JA (2000) Aerobic and anaerobic swimming performance of individual Atlantic cod. J Exp Biol 203:347–357

Roche DG, Binning SA, Bosiger Y, Johansen JL, Rummer JL (2013) Finding the best estimates of metabolic rates in a coral reef fish. J Exp Biol 216:2103–2110

Smith SDP, Mcintyre PB, Halpern BS, Cooke RM, Marino AL, Boyer GL, Buchsbaum A, Burton GA Jr, Campbell LM, Ciborowski JJH, Doran PJ, Infante DM, Johnson LB, Read JG, Rose JB, Rutherford ES, Steinman AD, Allan JD (2015) Rating impacts in a multi-stressor world: a quantitative assessment of 50 stressors affecting the Great Lakes. Ecol Appl 25(3):717–728

Tierney KB (2011) Swimming performance assessment in fishes. J Vis Exp 51:1–4

Tierney KB (2016) Chemical avoidance responses of fishes. Aquat Toxicol 174:228–241

Tix JA, Hasler CT, Sullivan C, Jeffrey JD, Suski CD (2016) Elevated carbon dioxide has limited acute effects on Lepomis macrochirus behaviour. J Fish Biol:1–22

Tix JA, Hasler CT, Sullivan C, Jeffrey JD, Suski CD (2017) Elevated carbon dioxide has the potential to impact alarm cue responses in some freshwater fishes. Aquat Ecol 51:59–72

Treanor HB, Ray AM, Layhee M, Watten BJ, Gross JA, Gresswell RE, Webb MAH (2017) Using carbon dioxide in fisheries and aquatic invasive species management. Fisheries 42:621–628

United States Army Corps of Engineers (2014) The GLMRIS report: Great Lakes and Mississippi River interbasin study. USACE, Washington, D.C.

Weiss LC, Pötter L, Steiger A, Kruppert S, Frost U, Tollrian R (2018) Rising pCO_2 in freshwater ecosystems has the potential to negatively affect predator-induced defenses in Daphnia. Curr Biol 28:1–6

Yoshikawa H, Kawai F, Kanamori M (1994) The relationship between the EEG and brain pH in carp, Cyprinus carpio, subjected to environmental hypercapnia at an anesthetic level. Comp Biochem Physiol 107(2):307–312

Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice-Hall, Englewood Cliffs, New Jersey